Replacement and Renumbering of Pages:

Please enter the attached 8 pages of printed Sequence Listing as new pages --102-109--.

Please renumber original pages "102-109" as pages --110-117--.

Please renumber the Abstract from page "110" to new page --118--.

REMARKS

Applicants respectfully request reconsideration and allowance of the claims as amended. Minor amendments have been made to the specification in order to correct typographical errors and informalities to which the Examiner objected. In addition three of the claims have been amended to include Sequence Listings. The printed Sequence Listing is identical to the Sequence Listing submitted in machine-readable form. This paper is submitted to comply with the United States Patent Office rules governing gene sequences. In no instance has new matter been added.

Rejection of Claims 1-26 under 35 USC § 112, Second Paragraph

In the December 6, 1995 Office Action, the Examiner expressed concern regarding the clarity and definiteness of the Applicants' use of the terms "stabilizing region" in claims 1, 3, and 4, use of the term "U6-type" in claims 2, 3, 7, 8, 15, 21, and 24, and failure to include sequence identification numbers in claims 16, 17, and 26.

(a) With regard to the use of the term "stabilizing region" the Examiner's attention is respectfully directed to page 28, line 4 through page 29, line 8 of the instant specification, where the term is clearly and concisely defined. This section outlines the general length of a stabilizing region, "between about 16 and about 50 nucleotides in length," and the number of mismatched bases tolerated in a stabilizing region, "[u]sually the mismatched bases are less than about 1 in 5

of the nucleotides in the complimentary regions of the predicted hairpin structure, and almost always less than about 1 in 4." In addition, according to this section of the specification, a stabilizing region of the instant invention "is preferably able to reduce the rate of intracellular degradation for the resulting RNA transcript" as measured by a RNA degradation assays like that of Example 11. Moreover, this section of the specification contains the absolute requirement that the RNA transcribed from the stabilizing region is "predicted to form a hairpin structure by a computer program that models and predicts secondary structure of RNA." Applicants submit that hairpin structures and computer programs which predict RNA secondary structure were both well known in the art at the time of filing, and exemplified in the instant application. For example, hairpin structures are shown in Figure 9 of the instant application, and the Martinez RNAFOLD algorithm for predicting RNA secondary structure is referenced in Example 8. Thus, Applicants submit that the specification provides a clear and concise definition of the term, "stabilizing region," so as to enable any person of skill in the art to make and use the constructs, oligonucleotide generators, and methods of the instant invention.

(b) With regard to the use of the term "U6-type" the Examiner's attention is respectfully directed to page 26, line 12 through page 27, line 14 of the instant specification, where the term is clearly and concisely defined. This section of the specification defines "U6-type" promoters as embracing the class of polymerase III, type III promoters described by Willis, I. (1993) FEBS 212: 1-11, which facilitate transcription by polymerase III, but are not themselves transcribed by the polymerase III enzyme. The Examiner's attention is particularly directed to the list promoters which are included in the designation of "U6-type RNA polymerase III promoters" page 26, lines 23-34 of the instant specification. These promoters include naturally-occurring U6 from higher eukaryotes, 7SK, H1 RNA genes, U3 snRNA genes in plants, and MRP genes. In addition, page 27, lines 4-14 describe how the recombinant U6-type promoters encompassed by the present invention can be constructed using the naturally-

occurring U6-type promoters listed above as a basis. Thus, Applicants submit that the specification provides a clear and concise definition of the term, U6-type, so as to enable any person of skill in the art to make and use the constructs, oligonucleotide generators, and methods of the instant invention.

(c) As described above, claims 16, 17, and 26 have been amended to include sequence identification numbers.

Since the terms "stabilizing region" and "U6-type" have been clearly and concisely defined in the application and claims 16, 17, and 26 have been amended to include sequence identification numbers, the Examiner is respectfully requested to withdraw his rejection of claims 1-26 under 35 USC § 112, second paragraph.

Rejection of Claim 1 under 35 USC § 102b

In the December 6, 1995 Office Action, the Examiner expressed a concern that the references by Jennings et al. and Sullenger et al. anticipate claim 1 of the instant invention.

However, neither article anticipates claim 1. In the case of Jennings, the construct does not contain the same type of stabilizing region as the construct of claim 1, and the promoter in the Jennings construct is in a completely different position relative to the stabilizing structure in the construct of claim 1.

The panhandle structure described on page 3043 of the Jennings article is not the same as the stabilizing region of claim 1. The stabilizing region of claim 1 would result in the formation of a "hairpin structure" at the 5' end of the resulting RNA transcript. See pages 28 and 29 of the specification for a description of the stabilizing region, and the "flanking stability region" of the "sample transcript" shown in figure 2. The panhandle structure of the Jennings transcript (p.3043, bottom of column 2) is formed by "base pairing of the 5' and 3' ends of the RNA"

transcript. The panhandle of Jennings would be a lariat type structure, not the hairpin that would result from the stabilizing region of claim 1.

Even, *arguendo*, if the stabilizing region of claim 1 did read on the "additional hexadecamer sequences" at the 5' and 3' of the Jennings RNA transcript, which it does not, the hexadecamer sequence is at the wrong location relative to the polymerase III promoter. Jennings makes use of an adenovirus VA RNA gene promoter, which is an example of a type II, polymerase III promoter. See Willis page 2, column 1. As a type II, polymerase III promoter, the VA1 promoter is intragenic; transcripts from this promoter include a transcribed copy of the promoter elements internally. Considering that the Jennings panhandle is formed by "base pairing of the 5' and 3' ends of the RNA," and the promoter elements are inside the RNA transcript, the 5' panhandle-forming hexadecamer in Jennings' system must be in the 5' direction from the promoter. However, claim 1 specifically states that the "promoter is in the 5' direction from the stabilizing region." Thus, the Jennings construct does not contain the same type of stabilizing region as the construct of claim 1, nor is the promoter in the Jennings construct in the same position relative to the stabilizing structure as the construct of claim 1.

Furthermore the Sullenger reference does not anticipate claim 1. At no point does Sullenger mention any type of stabilizing secondary structure in the RNA transcripts formed by his constructs. Claim 1 specifically contains a stabilizing region which is absent from any of the constructs which were produced or contemplated by Sullenger. In addition, Sullenger makes use of the t-RNA promoter which is again a type II, polymerase III promoter, which is transcribed and ends up in an internal portion within the RNA transcript. See Willis p. 2, column 1. Thus, even, *arguendo*, if there were a stabilizing region at the 5' end of one of Sullenger's transcripts, which there is not, Sullenger's constructs would not read on claim 1 for the same two reasons the Jennings constructs do not read on claim 1.

The Federal Circuit has held that prior art is anticipatory only if every element of the claimed invention is disclosed in a single item of prior art in the form literally defined in the claim. *Jamesbury Corp. v. Litton Indus. Products*, 756 F.2d 1556, 225 USPQ 253 (Fed. Cir. 1985); *Atlas Powder Co. v. du Pont*, 750 F.2d 1569, 224 USPQ 409 (Fed. Cir. 1984); *American Hospital Supply v. Travenol Labs.*, 745 F.2d 1, 223 USPQ 577 (Fed. Cir. 1984). Since one of the elements of claim 1 is either not present, in the case of the Sullenger article, or of the wrong type and in the wrong location, in the case of the Jennings article, neither of these references anticipate claim 1. The Examiner is, therefore, respectfully requested to withdraw his rejection of claim 1 under 35 USC § 102b.

Rejection of Claims 27 and 28 under 35 USC § 102a

The Examiner expressed concern that the Noonberg et al. BioTechniques reference anticipates claims 27 and 28. However, claims 27 and 28 find support in the parent application Serial No. 08/138,666. For example, triplex blotting techniques are described on page 3, lines 13–27, page 5, lines 27–28, page 6, lines 1–9, page 7, lines 17–28, and pages 15 through 18 of the parent application Serial No. 08/138,666. As a result, claims 27 and 28 are entitled to a priority of October 14, 1993, the filing date of the 08/138,666 application, and can not be anticipated by any art which was published in June of 1994. Therefore, the Examiner is respectfully requested to withdraw his rejection of claims 27 and 28 under 35 USC § 102a.

Rejection of Claims 2-26 under 35 USC § 103

In the December 6, 1995 Office Action, the Examiner expressed concern that the references by Jennings et al. and Sullenger et al. in view of the prior art knowledge of U6-type promoters as recounted on page 25, lines 15-24 would render claims 2-26 of the instant invention obvious. As the Examiner points out Jennings et al. and Sullenger et al. disclose the use of

polymerase III promoters to make recombinant constructs that produce oligonucleotides. In addition the Examiner states that these "vectors that produce oligonucleotides that are flanked by RNA polymerase III promoters and terminators." This is precisely the critical difference between the oligonucleotide generators of the instant invention and the constructs of Jennings, Sullenger, and the prior art. The oligonucleotide generators of the instant invention make use of a U6-type promoters, not the polymerase III, type II promoters utilized by Jennings and Sullenger. While the U6-type promoters are not transcribed, the polymerase III, type II promoters contain relatively large elements which are transcribed along with the desirable oligonucleotides. Thus, in the oligonucleotide generators of the instant invention, the resulting transcripts are not masked by these transcribed promoter elements or in danger of being preferentially transported from the nucleus by virtue of any t-RNA (Sullenger) or VA1 gene (Jennings) regulatory elements within the transcribed RNA. Indeed, neither of these references teach or suggest in any way that the use of a U6-type promoter would be advantageous. The Federal Circuit has held that "[o]bviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching, suggestion, or incentive supporting the combination." *ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed.Cir. 1984). There is no such teaching, suggestion, or incentive supporting the use of anything but polymerase III, type II promoters in Sullenger, nor anything but polymerase III, type I or II in Jennings.

Indeed, Sullenger et al. teaches away from the use of anything but tRNA promoters in these systems. In the introduction of the paper, Sullenger et al. report and contemplate the work of several individuals using studies utilizing various polymerase III promoter systems (p. 6512, column 2 through 6513, column 1). However, in their discussion of the potential benefits of introducing novel constructs which produce RNA oligonucleotides in cells, Sullenger comments that, "tRNA driven transcription systems may be particularly useful for introducing "mutations"

into the germ line, i.e., for generating transgenic animals or transgenic plants." (Sullenger et al. p. 6522) From the context of this statement it is clear that Sullenger, in his use of the term "mutations" in quotes, he means the introduction of constructs which produce RNA transcripts masking a particular phenotype, a desired result which is the subject of both the Sullenger article and the instant invention. By particularly recommending tRNA-driven transcription systems after considering a variety of other polymerase III and polymerase II promoter systems, Sullenger is teaching away from any construct which is not driven by a tRNA promoter.

In the case of Jennings et al., they only contemplate the use of polymerase III, type II promoters (the adenovirus VA1 promoter which is mentioned throughout Jennings), and polymerase III, type I promoters (5S rRNA gene promoters which are mention on page 3046 of the Jennings article). For a discussion of the classification of 5S rRNA gene promoters see Willis p. 2, column 1. There is no discussion of using any promoter which could be classified as a U6-type promoter in the Jennings article.

Since there is no suggestion in either Jennings or Sullenger that a U6-type or polymerase III, type III promoter could be used to generate antisense or triplex binding oligonucleotides in cells, these references do not support a *prima facie* holding of obviousness. Therefore, the Examiner is respectfully requested to withdraw his rejection of claims 2-26 under 35 USC § 103.

Rejection of Claims 27 and 28 under 35 USC § 103

In the December 6, 1995 Office Action, the Examiner expressed concern that the references by Jennings et al. and Sullenger et al. in view of Lyamichev et al. would render claim 1 of the instant invention obvious. As the Examiner points out, Jennings et al. and Sullenger et al. disclose nucleic acid blotting, while Lyamichev et al. discloses the electrophoresis of "triple stranded nucleic acids."

The filing of the priority document, application 08/138,666, represents the first disclosure of a triplex blotting assay, that is a blotting assay in which the triplex structure is formed between a single-stranded nucleic acid chain and a double-stranded nucleic acid chain after either one has been blotted or attached to a solid support. There is no evidence that a blotting assay which measures the formation of a triplex structure was ever performed or even contemplated. Indeed, it is the formation of the triplex structure after blotting which is a novel and inventive aspect of claims 27 and 28, as well as the aspect of these assays which make the methods of claims 27 and 28 such powerful tools in the search for novel triplex forming nucleic acid sequences.

While the Examiner is quite correct that both nucleic acids blotting assays and triple stranded nucleic acid structures were known in the art, the creation of a blotting assay that measures the formation of triple stranded nucleic acid chains after either the single- or double-stranded nucleic acid chain components has been attached to a solid support represents an inventive leap. Therefore, the triplex blotting assays of the present invention were not even obvious to try. The CCPA has held and the Federal Circuit has affirmed that "[o]bviousness does not require absolute predictability but a reasonable expectation of success is necessary." In re Clinton, 527 F.2d 1226, 1228, 188 USPQ 365, 367 (CCPA 1976); Amgen, Inc. v. Chugai Pharmaceutical Co. Ltd., 927 F.2d 1200, 18 USPQ2d 1016 (Fed.Cir. 1991). Indeed, the triplex structure is formed by an entirely different chemical interaction than the base pairing that results in the DNA and RNA duplexes of prior art blotting assays. The present invention makes use of the unique chemical interaction between a nucleic acid duplex and a third single strand of nucleic acids. Prior to the present invention there was no reliable evidence that stable triplex structures would form under the conditions of such an assay. Therefore, even, *arguendo*, if these assays had been obvious to try, which they were not, there is no information in the prior art which would reliably predict that triplex blotting would work. Thus, the Examiner is respectfully requested to withdraw his rejection of claims 27 and 28 under 35 USC § 103.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952**. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

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Respectfully submitted,

By: David L. Bradfute

David L. Bradfute
Registration No. 39,117
Filed under § 1.34(a)

Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, California 94304-1018
Telephone: (415) 813-5710
Facsimile: (415) 494-0792